

Magnesium Binding to Yeast Ribosomes

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Synopsis

This paper describes a theoretical and experimental analysis of the binding of magnesium ions to yeast ribosomes. In the theoretical considerations the interactions between charges located on a macroion are included. In the calculations these interactions result in a term, in which both the charge and the radius of the macroion are accounted for. It appears that on dissociation of the ribosomes both the charge and the radius change, but in such a way, that the term, which accounts for the electrostatic interactions, remains constant. As a consequence the dissociation can be neglected in the analyses of the binding experiments. Our experiments indicate that two binding reactions between ribosomes and magnesium ions occur. The endpoints of these reactions correspond to about 0.40 and 1.0 equivalent magnesium per ribosomal phosphate, respectively. The pK values are about 3.8 and 2.2, respectively. The experimental results indicate that the effect of monovalent cations can be explained as a pure ionic strength effect, though the binding of monovalent cations could not be excluded completely.

INTRODUCTION

In a previous paper¹ we described the dissociation and association behavior of yeast ribosomes. To interpret the results it appeared necessary to make two suppositions with regard to the binding of magnesium ions to yeast ribosomes. First it was proposed that monovalent cations are not bound to ribosomes. Second it was suggested that there are two classes of binding sites. Hence a more detailed study of the binding of magnesium ions to check the correctness of these suppositions seemed desirable.

In the literature only a few reports have been given on this binding.²⁻⁵ It has been found that the ionic strength has a marked effect on the amount of bound magnesium ions. At low ionic strength magnesium ions are bound very tightly. At high ionic strength the binding is much less tight. These results have been interpreted both as an ionic strength effect² and as a competitive effect.⁴ Finally it has been reported that the magnesium binding is affected by the pH. An increase of the pH results in a decrease of the amount of bound magnesium ions.^{2,3}

So far, the available experimental data have been treated only qualitatively. In the present paper an attempt will be made to treat the experimental data in a quantitative way, in terms of classes of binding sites, dissociation constants and ionic strength effect. First the theoretical

aspects will be given, followed by the experimental results. Finally the experimental results will be discussed in the light of the theoretical considerations.

MATERIALS AND METHODS

Isolation of ribosomes from baker's yeast (*Saccharomyces cerevisiae*), equilibrium dialysis and measurements of magnesium binding have been described elsewhere.¹ In all binding experiments the concentration of ribosomes was about 10 mg/ml.

In a number of experiments K_2SO_4 was added in such amounts, that in combination with the other electrolytes present in the solution, a constant ionic strength was obtained. Activities were calculated according to the Debye-Hückel approximation. Calculations of the binding curves were executed on the IBM system 360/50 with the use of a Fortran IV program.

THEORETICAL ASPECTS

When there is a reversible interaction between a macroion and small ions, e.g., magnesium ions, the simplest situation is the one in which all binding sites are identical and completely independent. In this case the relation between the fraction α of dissociated groups and the pMg is given by:

$$\text{pMg} = \text{p}K + \log [\alpha/(1 - \alpha)] \quad (1)$$

with $\text{pMg} = -\log [\text{Mg}^{++}]$ and $\text{p}K = -\log K$, where K is the dissociation constant. However, generally the identical binding sites interact with one another in such a way that binding at any site affects the binding affinity at all other sites. This phenomenon has to be attributed to the fact that the work required to dissociate a small ion from a macroion is a function of the charges located on the macroion. According to the theory of Linderstrøm-Lang⁶ this effect results in an extra term in eq. (1), which then becomes:

$$\text{pMg} = \text{p}K_{\text{int,Mg}} + \log [\alpha/(1 - \alpha)] - 0.868 w z_i Z \quad (2)$$

where Z is the total charge of the macroion; z_i is the charge of the small ion; $K_{\text{int,Mg}}$ is the intrinsic dissociation constant, i.e., the dissociation constant when the charge of the macroion is zero. w is the so-called electrostatic interaction factor, and the term $-0.868 w z_i Z$ can be considered as a measure for the extra work required to dissociate a small ion from a macroion because of its charge Z . This term accounts for the effect of the ionic strength on the interaction between charges located on the macroion. It should be noted that the Linderstrøm-Lang approach is an approximation, because the electrostatic interaction is considered as an effect of the total charge Z and not as the resultant of the interactions of the individual charges on the macromolecule. It is clear that w will be strongly dependent on the model chosen to represent the macroion, but calculations show that w is a constant for a chosen model as long as the temperature, ionic strength,

and conformation are constant. The latter condition, constant conformation, is not satisfied in our binding experiments, since at low magnesium ion concentration ribosomes dissociate into two subunits, according to the equation: $80\text{ S} \rightleftharpoons 60\text{ S} + 40\text{ S}$, where S is the Svedberg unit. To investigate the effect of the dissociation on w we have made some approximate calculations. In making these calculations, we have assumed that the ribosomes and their subunits are easily permeable for the solvent, including small ions and that they can be approximated by a sphere. In addition it was assumed that in view of the very high hydration, the volume of the ribosomal material (nucleic acids and proteins) is a negligible fraction of the particle volume. For such a model w can be calculated as has been done by Hermans and Overbeek.⁷ It is found that:

$$w = \frac{3\epsilon^2}{2DRkT} \left\{ \frac{1}{\kappa^2 R^2} - \frac{3}{2\kappa^5 R^5} [\kappa^2 R^2 - (1 + \kappa R)^2 e^{-2\kappa R}] \right\} \quad (3)$$

where ϵ is the proton charge; D is the dielectric constant of the solvent; R is the radius of the hydrated macroion; κ is the Debye-Hückel parameter; k is the Boltzmann constant, T is the absolute temperature.* Table I

TABLE I
Calculated Values of w and $^{4/3}\pi R^3 w$ as a Function of R According to Eq. (3)^a

| $R, \text{\AA}$ | $w \times 10^6$ | $^{4/3}\pi R^3 w \times 10^{21}$ |
|-----------------|-----------------|----------------------------------|
| 50 | 1609 | 8.42 |
| 75 | 605 | 10.69 |
| 100 | 287 | 12.03 |
| 125 | 157 | 12.87 |
| 150 | 95 | 13.46 |
| 175 | 62 | 13.86 |

* Numerical values: $\epsilon = 4.77 \times 10^{-10}$; $\kappa = 5.33 \times 10^6$ (corresponding with an ionic strength of 0.025); $D = 80$; $k = 1.37 \times 10^{-16}$; $T = 278^\circ\text{K}$.

shows values of w as a function of R , calculated by eq. (3). It is seen from this table that w strongly depends on the radius of the macroion. Since the radii of the subunits are smaller than those of the 80 S particle, w becomes larger on dissociation. However, the effect on $-0.868wz_1Z$ is at least partially compensated by a decrease of the charge Z , accompanying the dissociation. In eq. (2), Z can be replaced by $^{4/3}\pi R^3\rho$, where ρ is the charge density of the macroion. The quantity ρ is independent of the dissociation and association reactions. The third column of Table I shows the calculated values of $^{4/3}\pi R^3w$. The radius of the 80 S particle is about 155\AA . By using the known molecular weight ratio of the ribosomal subunits, viz., 2:1, and assuming that both subunits can be considered as nearly spherical particles, radii of about 135\AA and 110\AA can be calculated for the 60 S and 40 S particle, respectively. As can be seen from Table I

* The relation between the electrostatic free energy W_{el} , calculated by Hermans and Overbeek, and the electrostatic interaction factor w is given by $w = W_{el}/Z^2kT$.⁸

the value of $wZ = \frac{4}{3}\pi R^3 w\rho$ is within this region nearly independent of the dissociation and association reactions. The results of these calculations are strongly supported by the finding of Choi and Carr⁴ that the subunits of the ribosomes of *Escherichia coli* have identical binding curves. Hence it seems reliable to use eq. (2), in spite of the dissociation and association reactions. The charge density ρ is proportional to the charge per ribosomal phosphate, $r - 1$, where r is the amount of bound magnesium, expressed in equivalents per ribosomal phosphate.

It is well known that the ribosomal protein bears a small positive electrical charge at a neutral pH.⁹ For *E. coli* ribosomes Watson¹⁰ states that each ribosomal protein chain has an excess of about four positive charges at neutral pH. Since each yeast ribosome has about 150 protein chains,⁹ we assume that a yeast ribosome contains an excess of about 600 positive charges. As a yeast ribosome contains about 5000 phosphate groups the contribution of the ribosomal proteins must be about +0.1 per ribosomal phosphate. A very exact value of this charge is not important, because this charge is small as compared with the total charge. Hence the charge density of the ribosomes can be given by:

$$\rho = a(r - 1 + 0.1) \quad (4)$$

Replacing Z in eq. (2) by $\frac{4}{3}\pi R^3 \rho$ and subsequently substituting equation (4) we get:

$$\text{pMg} = \text{p}K_{\text{int,Mg}} + \log [\alpha/(1 - \alpha)] - 0.868wz_1^4 \frac{4}{3}\pi R^3 a(r - 1 + 0.1)$$

or:

$$\text{pMg} = \text{p}K_{\text{int,Mg}} + \log [\alpha/(1 - \alpha)] - A(r - 1 + 0.1) \quad (5)$$

As mentioned above, A is independent of the dissociation and association reactions.

The effect of the ionic strength on w is shown in Table II. As an example, the term $-0.868wz_1Z$ was calculated for the maximum value of Z , i.e., about -5000 , at a ionic strength of 0.005 . In this case $-0.868wz_1Z$ became about 31 . This value is unrealistically high. However, Schildkraut and Lifson¹¹ showed that the electrostatic potential ψ —and w is directly proportional with ψ —at the surface of a macroion is overestimated by the Debye-Hückel approximation, used in the calculation of ψ . They

TABLE II
Effect of the Ionic Strength on w , Calculated According to Eq. (3)^a

| Ionic strength | $w \times 10^5$ |
|----------------|-----------------|
| 0.1 | 26.4 |
| 0.05 | 50.6 |
| 0.025 | 95.3 |
| 0.010 | 208.3 |
| 0.005 | 357.1 |

^a Numerical values: $R = 150 \text{ \AA}$; other numerical data are the same as in Table I.

suggest that the "high local concentration of the counterions in the immediate vicinity of the fixed charges screen these charges from interaction with other fixed charges, to the extent that the system behaves as if the fixed ions carry a reduced charge. The notion of a reduced charge represents in a single parameter the deviation of the Debye-Hückel approximation from the true potential." According to the theory of Schildkraut and Lifson Z has to be replaced by λZ . λ appears to be constant over a wide range of salt concentrations. The results of Holcomb and Timasheff¹² suggest that λ is also independent of the charge of the macroion. Usually λ has a value in the range between 0.1 and 0.25. With a factor within this range more reasonable values of w are obtained.

When there are more classes of binding sites an equation like eq. (5) holds for each class. For instance, for two classes of binding sites, these equations are:

$$pMg = pK_{int,Mg,1} + \log [\alpha_1/(1 - \alpha_1)] - A(r - 1 + 0.1) \quad (6)$$

and

$$pMg = pK_{int,Mg,2} + \log [\alpha_2/(1 - \alpha_2)] - A(r - 1 + 0.1) \quad (7)$$

If the equations (6) and (7) are combined, we get:

$$\alpha_2 = \frac{K_{int,Mg,2}\alpha_1}{\alpha_1(K_{int,Mg,2} - K_{int,Mg,1}) + K_{int,Mg,1}} \quad (8)$$

Hence, with known values of $K_{int,Mg,1}$ and $K_{int,Mg,2}$, α_2 can be expressed in terms of α_1 . In the calculation procedure values for α_1 are chosen and the corresponding values of α_2 are calculated according to eq. (8). If the number of binding sites of each class is known (these numbers, expressed in equivalents magnesium per ribosomal phosphate, can be read from the experimental binding curve; see next section) the charge $r - 1 + 0.1$ can be calculated. Finally, with a known value of A , pMg is calculated according to eq. (6) or (7). $K_{int,Mg,1}$, $K_{int,Mg,2}$, and A are found by trial and error to fit the experimental data.

RESULTS

To examine the possibility that ribosomes have two classes of binding sites a number of binding experiments under a variety of conditions were performed. The results are shown in Figures 1-3. The experimental conditions are given in the legends of these figures. In nearly all curves an inflection point at about $r = 0.40$ is found. This can only mean that there are two classes of binding sites.

To examine the effect of the salt concentration on the amount of bound magnesium ions, binding experiments were carried out at ionic strengths of 10^{-1} , 2.5×10^{-2} , and 5×10^{-3} , respectively. To eliminate variations between different ribosome-populations the binding experiments were performed with the same ribosome-population. Figure 4 shows the charge of

the ribosomes as a function of pMg for the three different values of the ionic strength. The points are found experimentally. The lines are calculated by using $n_1 = 0.57$, $n_2 = 0.43$, (n_1 and n_2 are the number of binding sites of the two classes respectively, expressed in equivalents per ribosomal phos-

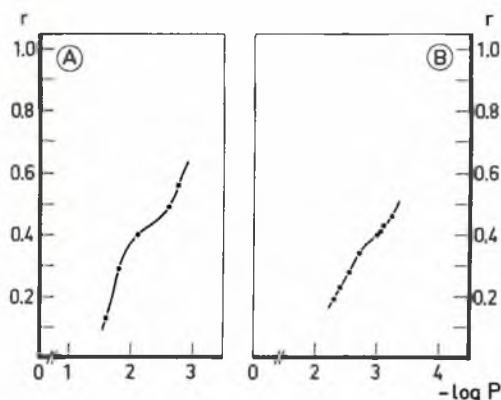


Fig. 1. Effect of the concentration P of the phosphate buffer ($\text{pH} = 7.4$) on Mg^{++} binding to yeast ribosomes at a constant free magnesium ion concentration: (A) $[\text{Mg}^{++}] = 2 \times 10^{-4}$; (B) $[\text{Mg}^{++}] = 10^{-5}$.

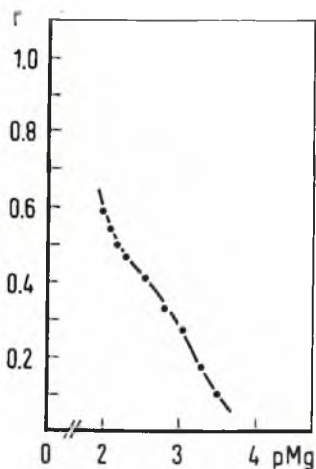


Fig. 2. Effect of pMg on Mg^{++} -binding to yeast ribosomes at a constant ionic strength of 0.1.

phate; hence $n_1 + n_2 = 1$) $\text{p}K_1 = 2.17$ and $\text{p}K_2 = 3.77$. The values of A are 0.01, 1.4, and 3.3, for a ionic strength of 10^{-1} , 2.5×10^{-2} , and 5×10^{-3} , respectively. It is seen that a reasonable fit with the experimental data is obtained.

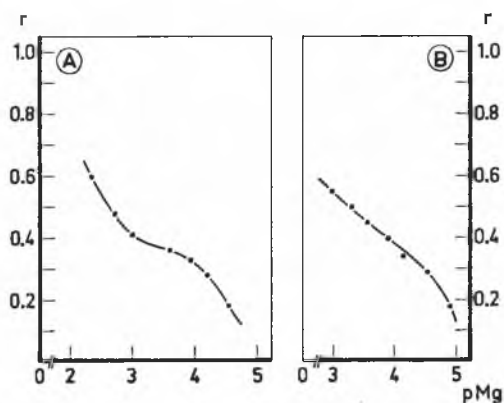


Fig. 3. Effect of pMg on Mg^{++} binding to yeast ribosomes at a constant phosphate buffer concentration: (A) 0.01 *m* phosphate buffer (pH = 7.4); (B) 0.002 *m* phosphate buffer (pH = 7.4).

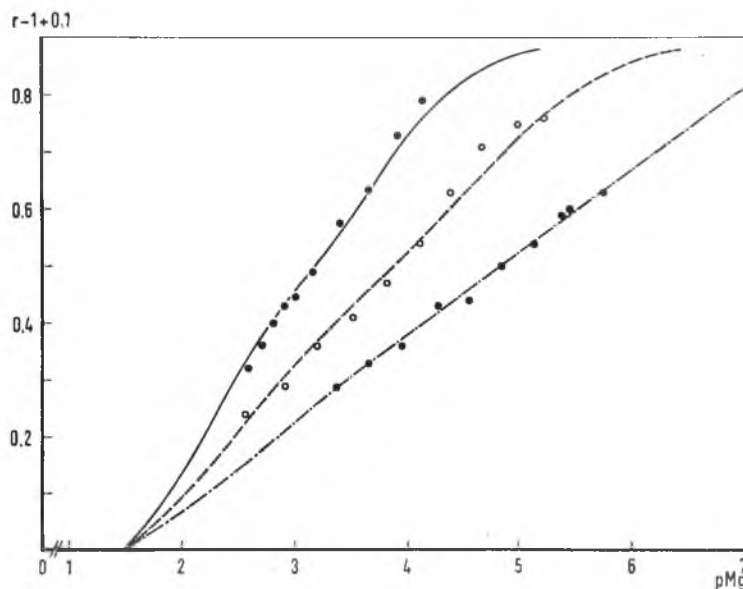


Fig. 4. Charge of the ribosomes, expressed in equivalents per ribosomal phosphate, versus pMg: (●, —) ionic strength is 0.1 and $A = 0.01$; (○, —) ionic strength is 2.5×10^{-2} and $A = 1.4$; (○, - - -) ionic strength is 5×10^{-3} and $A = 3.3$. The lines are calculated with $pK_1 = 2.17$, $pK_2 = 3.77$, $n_1 = 0.57$, and $n_2 = 0.43$. The points are found experimentally.

DISCUSSION

In this section some statements and results will be discussed in more detail.

Nature of the Binding of Magnesium Ions to Yeast Ribosomes

In the analysis of our binding experiments we have proposed that magnesium ions are bound to the ribosomal phosphate groups. However, with regard to the interaction of small ions to macroions two kinds of binding can be distinguished. First small ions can be bound to a well-defined site of the macroion. This type of binding is called site binding or specific binding. It is comparable with, for instance, the binding of H^+ ions to acetate ions. Second there can be binding due to the electrostatic potential of the macroion. This type of binding is called nonspecific binding or diffuse binding. It is comparable with the interaction of Na^+ ions with acetate ions. Experimentally it is almost impossible to distinguish site binding and diffuse binding, and in binding experiments both effects are measured together. However, the finding of Willemssen¹³ that at about pH 10 the binding of magnesium ions to polyadenylic acid and to polyuridylic acid is nearly identical, in spite of the quite different charge densities of the two polyelectrolytes, seems a strong indication that, at least in the presence of monovalent cations, the binding of magnesium ions to ribonucleic acids is a specific binding and that the contribution of nonspecific binding can be neglected. In the following sections we will understand by binding only site binding.

Two Classes of Binding Sites

From our experimental results evidence was presented that there are two classes of binding sites. At present it seems unlikely that these two classes have to be associated with a participation of the NH_2 groups of some bases in the interaction between rRNA and magnesium ions since spectrochemical studies indicated that the bases of polynucleotides are not involved in the binding of magnesium ions.¹⁴ Nevertheless the existence of two classes of binding sites includes that not all phosphate groups are identical. Indeed, in rRNA two conformations exist, viz., double-helical regions and single-stranded nonhelical regions to which most probably the ribosomal proteins are bound. The helical content of yeast rRNA has been estimated to be about 60%,⁹ hence the nonhelical content is about 40%. It is a striking fact that the two classes of binding sites contain about 60% and 40% of the phosphate groups, respectively. This suggests that the two classes of binding sites might reflect the two different conformations which occur in rRNA.

Effect of Monovalent Cations

We described an experiment concerning the binding of magnesium ions by yeast ribosomes at different salt concentrations (Fig. 4). From this experiment it will be clear that the ionic strength has a marked effect on the extent of magnesium binding. As already mentioned, it is still a matter of discussion whether this effect is a pure ionic strength effect or a competitive effect. It has been shown that a competitive effect results in

an apparent alteration of the intrinsic dissociation constant.¹⁵ In eq. (5) $K_{\text{int,Mg}}$ has to be replaced by

$$K_{\text{int,Mg}}[1 + (e^{-2wz_B Z}/K_{\text{int,B}})c_B]$$

where z_B is the charge of the cation B that can compete with magnesium ions; $K_{\text{int,B}}$ is the intrinsic dissociation constant of this cation, and c_B its concentration. For small values of Z , $K_{\text{int,Mg}}$ can be replaced by $K_{\text{int,Mg}}/[1 + (c_B/K_{\text{int,B}})]$. It will be clear that at about $Z = 0$ a competitive effect will not cancel, while under these conditions a pure ionic strength effect must cancel, since $0.868wz_i Z$ approaches zero. It appeared that a reasonable fit with the experimental data could be obtained by using the same pK values for the different salt concentrations, indicating that the effect of monovalent cations can be explained as a pure ionic strength effect and that the assumption of a competitive effect is not necessary. However, since the solution given is not unique a real competitive effect between potassium and magnesium ions can not be excluded. For a sound argumentation, the experimental curves of Figure 4 would have to be extended to higher magnesium concentration, and if then the three curves would intersect at about $Z = 0$ this would more clearly demonstrate the absence of a competitive effect. Unfortunately such an extension of the experimental curves is in practice impossible. At high magnesium ion concentrations the difference between the magnesium ion concentrations within the dialysis bag and those in the buffer is very small and hence the error in the experimental value of the amount of bound magnesium ions very large. In addition, at a low ionic strength the upper limit of the concentration of free magnesium ions is determined by the desired ionic strength.

The effect of monovalent cations has been explained as a competitive effect by Choi and Carr.⁴ These authors, in a study of magnesium binding to *E. coli* ribosomes, claim a weaker binding for monovalent cations than for divalent cations, but they pay no attention to the fact that a decrease of the magnesium binding with an increase in the concentration of monovalent cations might be caused by an alteration of the electrostatic interaction between the binding sites. Goldberg² found that for *E. coli* ribosomes the extent of magnesium binding at a free magnesium concentration of $6 \times 10^{-3}M$ is the same in the presence of 10^{-1} and $10^{-2}M$ potassium chloride, respectively. This finding seems not compatible with the idea of a competitive effect between potassium and magnesium ions. There is another argument that monovalent cations are not bound by polynucleotides. Ross and Scruggs calculated the effective charge per DNAP (phosphate groups of deoxyribonucleic acid) from electrophoresis experiments in the presence of TMA^+ (tetramethylammonium), K^+ , Na^+ , and Li^+ , respectively. Though the effects of these monovalent cations were not completely the same, it is remarkable that the charge per phosphate group did not alter over the range 0.05 – $0.4M$. Since in the case of binding the effective charge should decrease with increasing counterion concentra-

tion, this suggests in the authors' opinion that the monovalent cations are not bound at all. On the other hand the results of Ross and Scruggs are easily explained by the theory of Schildkraut and Lifson¹¹ as mentioned above. So in our opinion the conclusion that monovalent ions are not bound seems justified.

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